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ORIGINAL ARTICLE

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Does the presence of non-breeders enhance the fitness of breeders? An experimental analysis in the clown anemonefish *Amphiprion percula*

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Abstract The stability of animal societies depends on individuals' decisions about whether to tolerate or evict others and about whether to stay or leave. These decisions, in turn, depend on individuals' costs and benefits of living in the group. The clown anemonefish, *Amphiprion percula*, lives in groups composed of a breeding pair and zero to four non-breeders. To determine why breeders accept the presence of non-breeders in this species I investigated the effect of non-breeders on multiple components of the breeders' fitness. Non-breeders did not assist breeders in any obvious way. Experimental removal of non-breeders had no significant effect on the survival, growth, or reproductive success of breeders. Experimental removal of one of the breeding pair showed that non-breeders had little effect on the time taken for a widowed breeder to recommence breeding. The results indicate that the presence of non-breeders neither enhances, nor reduces, the fitness of breeders in *A. percula*. I suggest that non-breeders might modulate their effect on the fitness of breeders, either by reducing the costs they inflict or by increasing the benefits they provide, such that it just pays breeders to tolerate, rather than to evict, them. This study illustrates that animal societies can be stable even when some individuals gain nothing from the association.

Keywords Cooperative breeding · Helpers · Sociality · Reproductive skew · Marine fish

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Introduction

Many animal societies are composed of breeders and other individuals who either delay or completely forego their own reproduction. Such societies have generated interest amongst evolutionary biologists because of the potential for conflict and cooperation that lies within them (Keller and Reeve 1994). The stability of these societies depends on individuals' decisions governing group membership, which are intimately related to individuals' costs and benefits of living in the group (Danchin and Wagner 1997; Clutton-Brock et al. 1998; Buston 2003a). It is immediately apparent that breeders have higher reproductive success than non-breeders, and thus breeders might be expected to remain as long as their position remains favorable. It is usually less clear, however, why non-breeders should remain, and why breeders should tolerate the presence of non-breeders. The latter is especially true when non-breeders are potential competitors for reproduction (Reyer 1980; Taborsky 1985; Goldstein et al. 1998; Lundy et al. 1998).

There are several ways in which non-breeders can tip the balance of costs and benefits such that it pays breeders to accept them. First, non-breeders may enhance breeder survival (Reyer 1984; Woolfenden and Fitzpatrick 1984; Russell and Rowley 1988; Rood 1990), thereby increasing the breeders' lifetime reproductive success. Second, non-breeders may enhance the growth of breeders (Taborsky 1984; Clifton 1990), once again increasing the breeder's lifetime reproductive success. Third, non-breeders may enhance the number of offspring that breeders can produce each breeding season, either by increasing the number of offspring per breeding attempt (Brown et al. 1982; Taborsky 1984; Rood 1990; Emlen and Wrege 1991; Mumme 1992), or by increasing the number of breeding attempts (Brown and Brown 1981; Russell and Rowley 1988). Fourth, non-breeders may actually reduce these fitness components of breeders but the costs of their eviction may outweigh the costs of their toleration (Ens et al. 1992; Higashi and Yamamura 1993; Johnstone and Cant 1999).

Finally, the presence of non-breeders might benefit the breeders if they can serve as rapid mate replacements following the death of one of the breeders (Fricke 1979; Faulkes and Bennett 2001). The importance of this hypothesis is clearest when patches of inhospitable terrain separate groups, making successful dispersal between groups rare. Under such conditions, if a breeder dies and non-breeders are absent, the surviving breeder faces an extended period without reproducing while waiting for an immigrant mate to arrive. If a breeder dies and non-breeders are present, reproduction might recommence relatively rapidly. This hypothesis has been implicated in the evolution of sociality in anemonefishes (e.g. *Amphiprion akallopisos*, Fricke 1979) and in mole rats (e.g. *Heterocephalus glaber*, Faulkes and Bennett 2001), but its importance has not been evaluated in either case.

The 28 species of anemonefishes (Pisces: Pomacentridae) found on coral reefs throughout the Indo-Pacific (Allen 1972; Fautin and Allen 1992) present excellent opportunities for investigating the evolution of animal societies in a relatively unexplored taxon and environment. In Madang Lagoon, Papua New Guinea, groups of the clown anemonefish, *A. percula*, are confined to sea anemones (*Heteractis magnifica*) that provide the fish with food, oviposition sites, and protection from predators (Mariscal 1970; Fautin 1992; Elliott et al. 1995; Elliott and Mariscal 2001; Buston 2003a). Within each anemone there is a single group of *A. percula* composed of a breeding pair and zero to four individuals excluded from breeding (Fricke and Fricke 1977; Fricke 1979; Fautin 1992). These groups are not composed of close relatives (Buston et al., unpublished data). Within each group, the fish form a size-based dominance hierarchy (Fricke 1979; Buston 2003b); the female is largest, the male is second largest, and the non-breeders get progressively smaller. *A. percula* is a protandrous hermaphrodite (Fricke and Fricke 1977; Moyer and Nakazono 1978); if the female of a group dies, then the male changes sex and assumes the position vacated by the female, and the largest non-breeder from the anemone inherits the position vacated by the sex-changing male (Fricke 1979; Buston 2004). In *A. percula*, non-breeders do best by waiting within an anemone to inherit a breeding position, rather than dispersing and attempting to breed elsewhere (Buston 2004).

The question of whether or not anemonefish breeders benefit from the presence of non-breeders has been repeatedly raised but not experimentally tested (Fricke 1979; Taborsky 1984; Krebs and Davies 1991; Mitchell 2003). In this study, I propose five alternative hypotheses, each focusing on a different component of fitness, for why breeders might accept the presence of non-breeders in anemonefish. I also consider a null hypothesis in which non-breeders are neutral with respect to the fitness of the breeding pair. I conduct experimental tests of all the alternatives, using the well-studied population of the clown anemonefish *A. percula* in Madang Lagoon, Papua New Guinea.

Methods

Study population

I studied the clown anemonefish *A. percula*, in Madang Lagoon, Papua New Guinea (5°09′S, 145°48′E), from January 1997 to December 1997 (Buston 2002; see Buston 2003a for a more detailed description and justification of methods). I located 97 anemones (*H. magnifica*) on three reefs: reef 1, n=40; reef 2, n=31; reef 3, n=26. Each anemone was occupied by a single group of *A. percula*. Groups consisted of a breeding pair and zero to four non-breeders (mean number of individuals in each group ±SD=3.4±0.9, n=97). I recognized individuals $\geq$18 mm in standard length (SL) on the basis of natural variation in their color markings (Nelson et al. 1994; Elliott and Mariscal 2001; Buston 2003c), and I defined these individuals as residents or group members (n=334).

This study focused on 71 of the 97 groups, which were designated 'breeding groups' because they laid eggs in the first 2 lunar months of the study. I used lunar months as time steps in many of my analyses, because each lunar month is equivalent to a single breeding season (Ross 1978) and breeding occurs year-round with no apparent seasonality (Buston, unpublished data). To test my five hypotheses, I measured three components of breeder fitness: survival, growth, and reproduction.

Survival

I conducted a thorough census of each group every 1–2 days, for 10 lunar months (7 February–5 December), which enabled me to keep track of changes in group composition. If residents disappeared I concluded that they had died rather than migrated. I based this conclusion on three empirical results: (1) there is exceptionally high mortality of *A. percula* when they are beyond the periphery of their anemone (Mariscal 1970; Elliott et al. 1995); (2) no individuals that disappeared could be found after a thorough search of all the anemones in the study population, indicating that short distance migration did not occur (Buston 2003a); and (3) no migrants came into the study population from anemones outside of the study population, indicating that long distance migration did not occur (Buston 2003a).

Growth

In January, I captured all fish using hand nets and took them to the surface. There, I measured the SL of each individual to 0.1 mm. I used SL as my body size metric, rather than mass, because SL is unlikely to vary day to day. All fish survived these procedures without any sign of harm, and were returned to the anemone from which they were captured, where they remained. In December, I recaptured and re-measured all surviving fish, enabling me to estimate the growth (change in SL) for all surviving individuals.

Reproduction

I monitored the reproductive activity of breeders for the same 10 lunar months. Breeding was readily detectable; in the days before spawning the male selected a nest site next to the base of the anemone and cleared it of debris, and in the days after spawning the male spent much of his time tending the eggs. The exact age of the eggs was determined on the basis of their color (day 1 = orange; day 2 = orange-brown; day 3 = brown; day 4 = black eyes; day 5 = silver eyes; day 6 = silver eyes with pupils; day 7 = gold eyes with pupils). The eggs hatched after 7 days. To ensure a good estimate of the number of eggs hatched, I filmed each egg mass for 1 min on day 5 or day 6. Egg masses were filmed using Hi8 metal evaporated videotape and a Sony CCDTR700 8-mm videocamera in conjunction with an Amphibico Dive Buddy III underwater housing with dome port. I counted individual eggs from a frozen frame of the tape on a high-resolution monitor.

Justification for multivariate analyses and experimental manipulations

Within groups of *A. percula*, the size of the largest individual influences group size (Buston 2003b), group size correlates with anemone size (Fautin 1992), and anemone size correlates with the size of the largest individual (Fautin 1992). This triangle of correlates makes it difficult to investigate the effect of these parameters (female size, anemone size, and group size) on the fitness of the breeders, because colinearity among independent variables leads to difficulty in interpreting results (e.g. Mitchell 2003). Therefore, I used a combination of multivariate analyses and experiments specifically designed to determine the effect of non-breeders on the fitness of breeders (e.g. Brown et al. 1982; Leonard et al. 1989; Walters 1990; Emlen and Wrege 1991; Mumme 1992).

Breeder quality

Any effect of removing non-breeders could be obscured by variation in breeder quality and territory quality among groups. Therefore, I measured several attributes of breeders and territories so that they could be statistically controlled. To account for variation in breeder quality, I controlled for initial SL because SL may affect the number of eggs that female fish can lay (Wootton 1990), the effectiveness of male fish in caring for eggs (Lindstrom and Hellstrom 1993), growth (Wootton 1990), and survival (Booth 1995; Tupper and Boutilier 1995). When analyzing reproductive output I controlled for growth, and when analyzing growth I controlled for parental effort (female parental effort = number of clutches laid, male parental effort = number of clutches hatched), because these components may tradeoff against each other (Werner et al. 1983). Additionally, when analyzing survival or growth I controlled for the sex of the breeder.

Territory quality

To account for variation in territory quality, I controlled for four attributes of each territory: reef (identity), depth (m), anemone size (cm), and anemone saturation (degree). The location of the territory, given by reef and depth, has been shown to influence fitness in other species of coral reef fish (Jones 1986; Booth 1995; Beukers and Jones 1997; Hixon and Carr 1997). Anemone size reflects the size of the territory (Buston 2003a), and territory size could influence the fitness of breeders (Booth 1995; Beukers and Jones 1997). Anemone saturation is a measure of the density of fish in each anemone (Buston 2003a), and local density might influence the fitness of breeders (Tupper and Boutilier 1995; Hixon and Carr 1997).

Statistical analyses

I investigated the effect of removing non-breeders on components of breeder fitness using multivariate analyses (SAS GENMOD where the dependent variable was the occurrence of mortality; SAS PROC MIXED where the dependent variable was breeder growth or reproduction). When there was more than one measure of the dependent variable per group (e.g. survival of males and females), I entered group identification number as a random effect. This approach enabled me to test for effects of multiple independent variables, while controlling for the lack of independence among individuals within the same group. I removed control variables from the analysis in a backwards stepwise fashion if they did not have a significant effect ($P>0.05$), which enabled me to avoid some of the problems associated with colinearity among independent variables. The effect of the non-breeder removal was always retained in the analysis.

Experimental tests of hypotheses

Hypothesis 1: survival benefit

This hypothesis predicts that breeders will suffer higher mortality in groups from which all non-breeders are removed, than in control groups in which non-breeders are present. I removed all non-breeders from 14 breeding groups, 4 lunar months after the beginning of the study. Manipulated groups were randomly selected from the pool of breeding groups, on reefs 1 and 2. Control groups (n=41) were the remainder of the pool of breeding groups on reefs 1 and 2. I monitored breeder disappearance from all groups for 6 lunar months following the removals. I compared breeder mortality in 14 manipulated groups with that in 41 control groups.

Hypothesis 2: growth benefit

This hypothesis predicts that breeders will grow less rapidly in groups from which all non-breeders are removed, than in control groups in which non-breeders are present. I used a subset of the manipulated (non-breeders removed) and control groups (non-breeders present) used to test H1 (hypothesis 1). I restricted analysis to 49 breeding groups from which neither member of the breeding pair disappeared. I measured breeder growth over the entire 10-month study period. I compared the growth of breeders in 13 manipulated groups with that in 36 control groups.

Hypothesis 3: reproductive benefit

This hypothesis predicts that breeders will hatch fewer eggs per breeding season in groups from which all non-breeders are removed, than in control groups in which non-breeders are present. More powerfully, it predicts that the number of eggs hatched per lunar month will drop following non-breeder removal in manipulated, but not in control, groups. Although reproductive success may have multiple components (e.g. number of eggs per clutch, number of clutches, and hatching success) the number of eggs hatched per breeding season captures all of these components. I used the same set of manipulated (non-breeders removed) and control groups (non-breeders present) used to test H2. I compared the number of eggs hatched per lunar month in 13 manipulated groups with that in 36 control groups, in the months before and after the removal of non-breeders.

Hypothesis 4: eviction cost

Under this hypothesis, non-breeders are only tolerated because the costs of their eviction outweigh the costs of their toleration. This hypothesis makes two predictions: (1) that breeders will have higher fitness in groups from which non-breeders are removed, than in control groups in which non-breeders are present; and (2) that eviction is costly. I tested prediction no. 1, using the same set of manipulated (non-breeders removed) and control groups (non-breeders present) used to test H1–3. I compared manipulated groups to control groups to evaluate whether the breeders incurred survival (n=14 manipulated, n=41 control), growth (n=13 manipulated, n=36 control), or reproductive (n=13 manipulated, n=36 control) costs from the presence of non-breeders.

Hypothesis 5: mate-replacement benefit

This hypothesis predicts that widowed breeders will take longer to recommence breeding in groups from which non-breeders are removed, than in control groups in which non-breeders are present (Fig. 1). I conducted two experimental removals that enabled me to estimate all of the parameters necessary to evaluate this hypothesis from the females' perspective: (1) a removal of all non-breeders, to estimate the time for recruitment to occur in the absence of non-

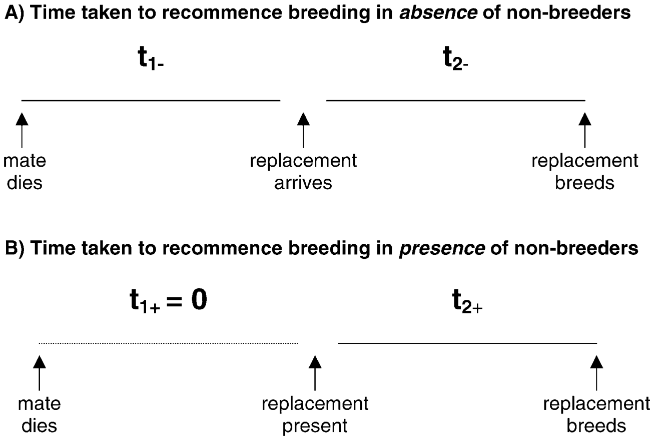
A) Time taken to recommence breeding in *absence* of non-breeders
t1-
t2-
mate dies
replacement arrives
replacement breeds
B) Time taken to recommence breeding in *presence* of non-breeders
t1+ = 0
t2+
mate dies
replacement present
replacement breeds

Fig. 1 The rapid mate-replacement hypothesis. The absolute benefit accrued from retaining non-breeders depends on the difference in the amount of time taken to recommence breeding **A** when non-breeders are absent (–), and **B** when non-breeders are present (+). When non-breeders are absent, the time taken to recommence breeding depends both on the time taken for a new individual to arrive (t_{1-}) and the time taken for the new individual to start functioning sexually (t_{2-}). When non-breeders are present, the time taken to recommence breeding only depends on the time taken for the resident non-breeder to start functioning sexually (t_{2+})

breeders (t_1; Fig. 1); and (2) a male removal, to estimate the time for a non-breeder to start functioning as a male (t_2; Fig. 1).

I conducted a male removal, rather than a female removal, because the benefit that females might accrue was expected to be larger and more detectable than the benefit that males might accrue. When the male dies, the female is ready to recommence breeding immediately, and the time interval before she recommences breeding depends solely on how long it takes the male's replacement to start functioning sexually. When the female dies, the male is not ready to recommence breeding immediately, because he must undergo sex change before he can begin breeding as a female. If the time required for the male to change sex is greater than the time required for the largest non-breeder to become functionally male, then this will erode the magnitude of the absolute benefit a male might gain from retaining a non-breeder as a mate replacement.

Non-breeder removal: estimation of the time required for a larva to recruit. This hypothesis predicts that the time taken for recruitment to occur will be large, thus generating a benefit of retaining a non-breeder. I used the same set of manipulated (non-breeders removed) groups that were used to test H2 and H3 (n=13). I monitored the time taken for recruitment to occur (i.e. the time taken for a larva to settle and grow to 18 mm SL; Buston 2003a) in the 6 months following non-breeder removal.

Male removal: estimation of the time required for a non-breeder to mature. This hypothesis predicts that the time taken to recommence breeding will be shorter when the mate replacement is initially larger, thus generating a benefit of retaining a large non-breeder. I removed males from 16 groups, all of which had resident non-breeders (non-breeders $\geq$18 mm SL; Buston 2003a), 1 lunar month after the beginning of the study. Manipulated groups were those that had bred, and thus were known to have a functioning female, during the first lunar month of the study, on reef 3. I measured the time taken for the female to recommence breeding with her new mate, and tested for an effect of the initial size of the mate replacement on the time taken to recommence breeding.

Results

Absolute fitness effects of retaining non-breeders

Hypothesis 1: survival benefit

Only 3 breeding females and 4 breeding males died in all 55 groups (14 manipulated; 41 controls). The removal of non-breeders did not have a significant negative association with the probability of breeder mortality (Chi-square test: df=1; χ^2=0.0374, P=0.8467; Table 1). Indeed, none of the independent variables investigated had a significant association with the probability of mortality.

Hypothesis 2: growth benefit

Breeders grew very little over the 10-lunar-month study period (mean change in SL±SD=1.5±1.2 mm, n=98). The removal of non-breeders did not have a significant negative effect on growth (Table 2, Fig. 2). The growth of breeders was, however, related to their sex (females grew more than males) and negatively related to both initial SL and parental effort (Table 2).

Hypothesis 3: reproductive benefit

Each breeding pair hatched several hundred eggs each month (mean±SD=414±300 eggs hatched/lunar month, n=49). The removal of non-breeders did not have a significant negative effect on the number of eggs hatched, indicated by the non-significant interaction between 'manipulation' and 'before/after' (Table 3, Fig. 3). The number of eggs hatched was, however, negatively related to the growth of the male (Table 3). Also, the number of eggs hatched was related to the main effect of 'manipulation' (Table 3, Fig. 3), indicating that the manipulated

Table 1 Survival and disappearance of breeders, as a function of the experimental removal of non-breeders (P>0.05). A logistic regression analysis that investigated the effect of multiple independent variables found no significant predictors of breeder mortality

Breeders	Survived	Disappeared
Non-breeders present	77	5
Non-breeders removed	26	2

Table 2 Growth of breeders on control and removal anemones. Summary of the results (fixed effects) of a mixed model analysis that investigated the effect of multiple independent variables. *ndf* Numerator degrees of freedom, *ddf* denominator degrees of freedom

Effect	*ndf*	*ddf*	*F*	*P*
Sex	1	46	24.22	0.0001
Parental effort	1	46	14.26	0.0005
Initial standard length	1	46	12.31	0.0010
Manipulation	1	46	0.60	0.4428

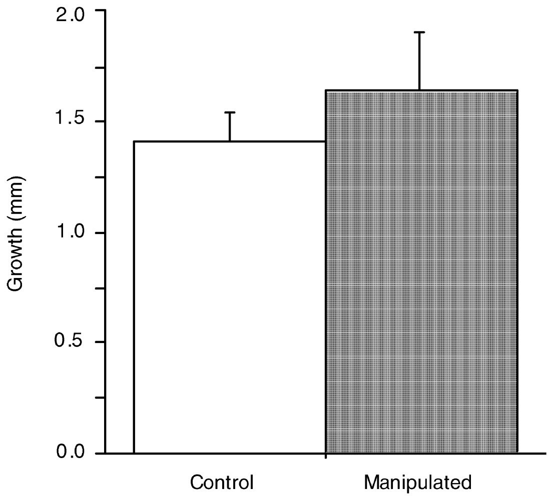

Fig. 2 Growth of breeders, as a function of the experimental removal of non-breeders ($P>0.05$). *Horizontal lines* represent the least squares mean estimate and *bars* are standard errors of this estimate

Table 3 Eggs hatched by breeders on control and removal anemones, before and after the manipulation was carried out. Summary of the results (fixed effects) of a mixed model analysis that investigated the effects of multiple independent variables. *ndf* Numerator degrees of freedom, *ddf* denominator degrees of freedom

Effect	*ndf*	*ddf*	*F*	*P*
Male growth	1	341	12.97	0.0004
Manipulation	1	341	4.94	0.0269
Before/after	1	341	0.29	0.5934
Manipulation × before/after	1	341	0.81	0.3193

groups were in some way different from the control groups even though they were randomly chosen.

Hypothesis 4: eviction cost

The removal of non-breeders did not have a significant positive effect on survival (Table 1), growth (Table 2, Fig. 2), or number of eggs hatched (Table 3, Fig. 3). These results indicate that breeders do not suffer fitness costs as a result of the presence of non-breeders. Given that there were no costs to toleration of non-breeders it was not necessary to evaluate the costs of eviction.

Hypothesis 5: mate-replacement benefit

Non-breeder removal: estimation of the time required for a larva to recruit. Recruitment occurred in all of the anemones from which non-breeders were removed. The mean time taken for recruitment to occur was 2.3±1.8 lunar months (n=13). The remaining anemone was excluded from the analysis because of the disappearance of the breeders.

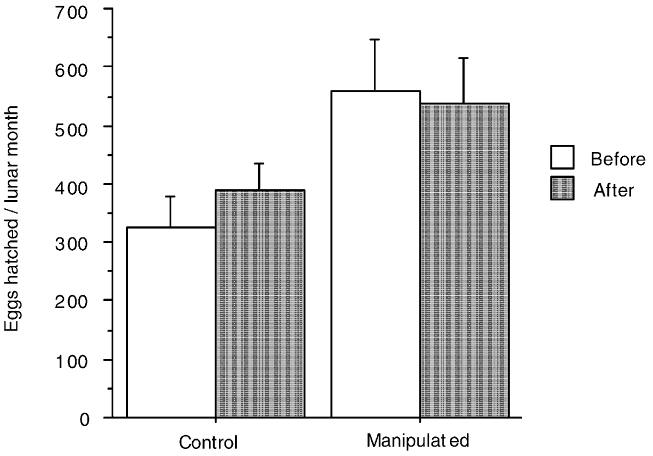

Fig. 3 Eggs hatched per lunar month by breeders, as a function of the experimental removal of non-breeders and the time relative to the experimental manipulation (*before* prior to removal, *after* post removal; $P>0.05$). *Horizontal lines* represent the least squares mean estimate, and *bars* are standard errors of this estimate

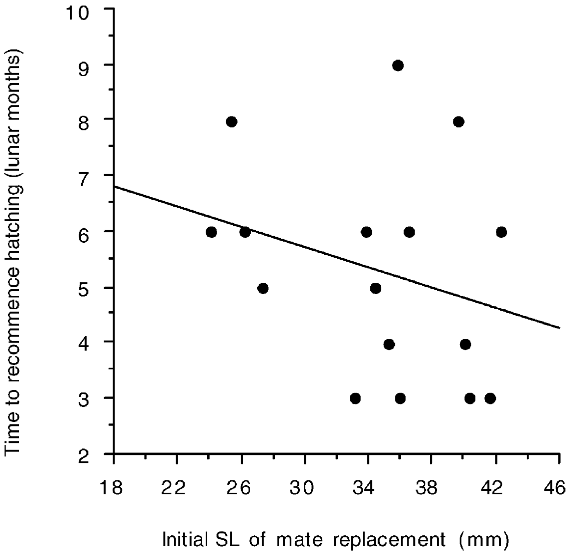

Fig. 4 Time for hatching to recommence following the removal of the male (t_2; Fig. 1), as a function of the initial standard length (SL) of the mate replacement ($P>0.05$). The *fitted line* is a linear regression ($y=8.48-0.92x$, n=16)

Male removal: estimation of the time required for a non-breeder to mature. Breeding recommenced in all 16 groups from which males were removed. The mean time taken for hatching to recommence was 5.3±1.9 lunar months (n=16). There was no significant relationship between the initial SL of the mate replacement and the time it took for hatching to recommence (df=1, F=1.21, P=0.29; Fig. 4).

Summary of hypothesis 5

The absolute benefit accrued by retaining non-breeders as mate replacements depends on the difference in the amount of time taken to recommence breeding when non-breeders are present compared to when they are absent (Fig. 1). The expected time for a mate replacement to arrive in the absence of non-breeders (t_{1-}) was 2.3 lunar months, the mean of the recruitment times. The expected time for the mate replacement to breed (t_{2+}) was not contingent on the initial size of the mate replacement (Fig. 4), suggesting that $t_{2-} \approx t_{2+}$. Thus the absolute benefit accrued by a female from retaining a single non-breeder as a mate replacement was 2.3 lunar months of reproduction, i.e., 2.3 breeding seasons.

Relative fitness of breeders with and without non-breeders

The relative fitness of the two strategies, accepting and rejecting non-breeders, will depend on their effects on each component of fitness summed across the lifetime of individuals. The only detectable effect that non-breeders had on the fitness of breeders was found in the context of the mate-replacement hypothesis (H5). Females gained an average of 2.3 lunar months of reproduction from retaining a single non-breeder as a mate replacement.

Setting the relative fitness of females that accept non-breeders, $W_{(+)}$, to 1.0, the fitness of females that reject non-breeders, $W_{(-)}$, can be estimated:

$$W_{(-)} = \left(\frac{T - NA}{T} \right) \tag{1}$$

where T is the expected breeding tenure of females (lunar months), A is the absolute benefit of retaining a non-breeder as a mate replacement (lunar months), and N is the expected number of mate replacements that females will require during their tenure.

The expected breeding tenure of females (T) will be the reciprocal of their instantaneous mortality rate. Only three breeding females and four breeding males died in 10 lunar months, and there was no detectable sex difference in the mortality rate (Table 1). The per lunar month mortality rate of breeders (female and male) was estimated to be $[(\ln 103/110)/10]=6.5\times10^{-3}$. Thus the expected tenure of breeding females (T) was 152 lunar months (approximately 12 years). This estimate may seem extraordinary for such a small fish, but it is plausible given estimates of longevity for other anemonefishes (13 years for *A. clarkii*, Moyer 1986; 18 years for *A. frenatus* and 18+ years for *A. perideraion*, Fautin and Allen 1992).

The expected number of mate replacements that females will require during their tenure (N) will be dependent on the relative mortality rates of females and males. Let f= the probability that females will die in 1 lunar month, and m= the probability that males will die in 1 lunar month. The probability that females will use one mate replacement during their tenure is $(1-f)(m)(T)$, and the probability that they will use n replacements is $(1-f)(m^n)(T)$. Thus, the expected number of mate replacements that females will require is:

$$\sum_{n=1}^{\infty} (1-f)(m^n)(T) \tag{2}$$

Given that the probability of breeders dying in 1 lunar month $f=m=6.5\times10^{-3}$, and the expected tenure of breeding females is 152 lunar months, the expected number of mate replacements that females will require (N) is approximately one.

The relative fitness of females without non-breeders was estimated by substituting values of T, N, and A into Eq. 1:

$$W_{(-)} = \left(\frac{T - NA}{T} \right) = \left(\frac{152 - (1)(2.3)}{152} \right) = 0.98 \tag{3}$$

Females that accept a single non-breeder are estimated to have a relative fitness approximately 2% higher than females that do not accept non-breeders.

Discussion

Effects of non-breeders

I examined the effect of non-breeders on the fitness of breeders in the clown anemonefish *A. percula*, in Madang Lagoon, Papua New Guinea. I used experimental removals of non-breeders and breeders, in conjunction with multivariate analyses, to test five alternative hypotheses. I found that the presence of non-breeders had little if any effect on breeder fitness. There were no detectable positive effects of non-breeders on the survival (H1), growth (H2), or reproduction (H3) of breeders; and I observed no behaviors that would suggest non-breeders were assisting in any way. Equally, breeders suffered no detectable negative effects from the presence of non-breeders (H4), which might have indicated that non-breeders were only tolerated because of high costs of eviction. The only effect of non-breeders occurred in the context of them serving as rapid mate replacements for widowed breeders (H5): females accrued a 2% gain in relative fitness by retaining a single non-breeder as a mate replacement.

Given that rapid mate replacement is the standard adaptive explanation for why anemonefish breeders accept non-breeders (Fricke 1979; Fautin and Allen 1992), the small magnitude of the benefit was surprising. The mate-replacement benefit was small for three reasons. First, it took little time for a mate replacement to arrive in the absence of non-breeders, and once a mate replacement was present there was no detectable effect of its initial size on the time taken to recommence breeding (Fig. 4), which meant that females without non-breeders only lost a little more breeding time than females with non-breeders (Fig. 1). Second, the mortality rates of males and females were similar (Table 1), so the number of times that females expected to utilize mate replacements was small (Eq. 2). Third, the expected breeding tenure of females was long relative to the amount of breeding time they lost by not having non-breeders. The mate-replacement benefit could be larger if there was a minimum age of first reproduction, because this would cause an additional delay for females that relied on new recruits as mate replacements, but this possibility could not be evaluated with the available data.

Even if the mate-replacement benefit was larger, it could not explain why breeders tolerated the presence of more than a single non-breeder. In Madang Lagoon, groups consisted of a breeding pair and zero to four non-breeders (mean number of individuals in each group ±SD=3.4±0.9, n=97). Yet, the expected number of mate replacements that females would utilize during their tenure was only one. In the unlikely event that more than one mate replacement was required, the expected time before females would require the second mate replacement was 10 years and within this time a new recruit certainly would have arrived and attained the minimum age for first reproduction. Thus females are unlikely to ever benefit from the presence of more than one non-breeder in the anemone. Because up to four non-breeders may be present in an anemone, the mate-replacement hypothesis cannot completely explain why breeders accept non-breeders in *A. percula*.

Comparison with other species in the genus offers further evidence that the mate-replacement hypothesis does not provide a complete explanation for why breeders accept non-breeders. In other species of anemonefish (e.g., *A. clarkii*), non-breeders are tolerated within territories, even though mate replacements are often migrants from other anemones (Ochi 1989; Hattori 1994). In these populations the time for a mate replacement to arrive in the absence of non-breeders tends toward zero (i.e. $t_{1-} \approx t_{1+}$), because migrations occur as soon as breeding vacancies arise. Also, in these populations the time for the immigrants to start functioning sexually is the same in anemones with and without non-breeders (i.e. $t_{2-} \approx t_{2+}$), because it is the largest non-breeders from the population as a whole (rather than from within the focal anemone) that serve as mate replacements. I conclude that rapid mate replacement is not the major explanation for why breeders accept non-breeders in the anemonefishes.

Caveats to the conclusion

I conclude that non-breeders do not enhance the fitness of the breeding pair, but this conclusion comes with three caveats. The first is that spatial and temporal variability within my study population might lead to low power of some of the analyses. Low power was of greatest concern for the test of the survival benefit hypothesis (Table 1), but subsequent approximations of power revealed it to be over 0.95, indicating that the probability of rejecting the null hypothesis if it were in fact false was close to one (Zar 1984). Low power was not of such great concern in tests of the growth or reproductive benefit hypotheses, where it can be concluded that any effect of non-breeders is trivial relative to the effect of other factors (Tables 2 and 3).

The second caveat is that spatial and temporal variability beyond my study population might mean that non-breeders enhance breeder fitness in some years or locations. The possibility that the effect of non-breeders varies among populations applies to all investigations of this type. However, this study is remarkable for monitoring nearly 100 groups for 10 breeding seasons, and it complements two other studies of anemonefishes [*A. akallopisos* at Aldabra Atoll (Fricke 1979), and *A. ocellaris* at Bunaken Island (Mitchell 2003)] that have not documented any effect of non-breeders on the fitness of breeders.

The final caveat is that I investigated direct effects of non-breeders on breeder fitness, but not indirect effects mediated through effects on anemone fitness. Non-breeders might indirectly enhance breeder fitness if (1) they enhance some component of anemone fitness (survival, growth, or reproduction), and (2) the breeders benefit from enhanced anemone fitness. This hypothesis was considered to be somewhat unlikely because no anemone mortality, growth, or reproduction was detected during my studies (Buston, unpublished data).

However, non-breeders might enhance the fitness of the anemone. The presence of anemonefish (*A. melanopus*) does enhance the survival of anemones (*Entacmaea quadricolor*, Fautin and Godwin 1992), though no effect of the number of fish on anemone survival has been documented. The number of anemonefish (*A. chrysopterus*) present on anemones (*H. magnifica*) can be positively related to anemone growth and/or reproduction (Schmitt and Holbrook 2003), though it is unclear whether this results from new recruits moving to high quality anemones or from the fish enhancing anemone fitness.

Further, attributes of the anemone can enhance the fitness of the fish. Anemone survival is crucial for the survival of the fish (Mariscal 1970; Elliott et al. 1995; Buston 2003a). Anemone size is positively related to the growth of resident fish (Buston 2002), and large fish might be able to raise more offspring (Fricke 1979). In summary, a direct or an indirect effect of non-breeders on the fitness of breeders might be detected in a longer study involving more groups, but currently it is simplest to conclude that non-breeders have no effect on the fitness of breeders.

Neutral non-breeders

My results support the null hypothesis, that the fitness of breeders is unaffected by the presence of non-breeders. This neutrality could be a simple effect of the ecology of the system, or it could be that non-breeders modify their behavior to maintain neutrality. In *A. percula* it seems more likely that non-breeders are actively maintaining their neutrality. Just by being present in the anemone non-breeders are potential competitors for reproduction, and they must do something to mitigate this effect given that breeders can evict them (Buston 2003a). Elsewhere, I demonstrate that non-breeders avoid becoming actual competitors for reproduction, and thereby avoid eviction, by modifying their growth and remaining small (Buston 2003b). This growth restraint is best viewed as a form of peaceful cooperation (avoiding inflicting a cost), rather than the more usual helpful cooperation (providing a benefit), as payment for being allowed to stay in the territory (Gaston 1978; Emlen 1991; Balshine-Earn et al. 1998; Kokko et al. 2002).

Conclusion

In *A. percula*, breeders tolerate the presence of multiple non-breeders because they are unaffected by their presence. Non-breeders provide no benefits in terms of survival, growth, reproduction, or mate replacement. Non-breeders are potentially costly competitors for reproduction, but they avoid becoming actual competitors for reproduction by modifying their growth and remaining small (Buston 2003b). Non-breeders might be modulating their impact on the fitness of breeders such that it just pays the breeders to tolerate them rather than to evict them (Kokko et al. 2002). This study demonstrates that animal societies can be stable even when some individuals do not benefit from the presence of others.

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